

REMARKS

Claims 62 and 69-74 have been canceled. Claims 1, 18, 58, 60 and 63-67 have been amended. New claims 75-87 have been added. The amended and new claims are supported throughout the application as filed, e.g., at, page 17, line 29 to page 18, line 29; page 28, line 26 to page 29, line 5; page 3, lines 18-28; page 18, line 21 to page 19, line 8, and by the original claims. No new matter has been added. Upon entry of this amendment, claims 1, 6, 7, 13-23, 53-61, 63-68 and 75-87 will be pending and under examination.

Claim Objection

Claim 18 is objected to as redundant for reciting "skin proliferation in the skin." Claim 18 has been amended to correct this redundancy, as suggested by the Examiner.

Rejections Under 35 U.S.C. § 112

Enablement

Claims 1, 13-23, amend 53-74 are rejected as "containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." The present claims are directed to a method of treating a subject having a disorder characterized by unwanted cell proliferation. The method includes administering a TSP-2 being at least 90% identical to SEQ ID NO:2 or a fragment thereof having the ability to inhibit endothelial cell migration. According to the examiner,

...the specification does not give any guidance to which TSP-2 fragments will exhibit the biological activities as the claimed, or any guidance as to which regions of amino acid sequence are responsible for biological activity and thus, must be preserved so the molecule will function as claimed, or how to make and select for such molecules."

This rejection has been met, in part, by narrowing claim 1 (from which the other claims depend) to recite a TSP-2 at least 90% identical to SEQ ID NO:2 or a fragment thereof having a particular biological activity, namely the ability to inhibit endothelial cell migration. New claims 85-87 add additional limitations on the structure of the TSP-2. This rejection is respectfully

C3
Figure 6 is a graph showing the migration of human dermal microvascular endothelial cells (HDMEC) and the effect of TSP-1 (T1) or TSP-2 (T2) binding of the CD36 receptor on the migration of these cells. HDMEC were incubated alone (C), in the presence of TSP-1 (T1) or TSP-2 (T2), or in the presence of TSP-1 (T1) or TSP-2 (T2) in the presence of an anti-CD36 antibody (36).

Please replace the paragraph beginning at page 26, line 17, with the following rewritten paragraph:

Sub D1
C4
Figure 7 is a graph showing the effect of HDMEC migration in the presence of various synthetic TSP-2 derived peptides. Peptides 1, 2, 3 and 4 (P1, P2, P3, P4) (SEQ ID NOs:1-4, respectively) were derived from the procollagen domain of TSP-2; peptide 7 (P7) (SEQ ID NO:10) was derived from the first type 1 repeat of TSP-2.--

In the claims:

Cancel claims 62 and 69-74.

For the Examiner's convenience, all the pending claims, whether or not amended, are reproduced below. Please amend claims 1, 18, 58, 60 and 63-67 as follows.

- Sub D2*
C5
1. (Amended) A method of treating a subject having a disorder characterized by unwanted cell proliferation, the method comprising administering to the subject a TSP-2 comprising an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:2, or a fragment thereof capable of inhibiting endothelial cell migration.
6. (Reiterated) The method of claim 1, wherein the fragment comprises the sequence of SEQ ID NO:10 (WSPWAEW).
7. (Reiterated) The method of claim 1, wherein the fragment consists of the sequence of SEQ ID NO: 10 (WSPWAEW).

traversed with regard to the presently pending claims. Combined with the high level of skill in the art, the specification provides sufficient guidance for a skilled artisan to practice the full scope of the claimed methods without undue experimentation.

In the present case, the TSP-2 sequence is known, and molecular biology techniques for making TSP-2 fragments as recited in the claims are routine in the art. In addition, the specification provides guidance for making variants that are highly similar in sequence, as required by the claims, e.g., TSP-2 having conservative amino acid substitutions (see, e.g., pages 48-49). The specification describes the inhibition of tumor growth by TSP-2 in at least 2 different in vivo models: inhibition of squamous cell carcinoma cell grafts (A431 cells) and malignant melanoma cell grafts (MeWo cells) in mice (see, e.g., page 33, line 17 to page 34, line 19, and Figure 3A and 3B). The specification clearly shows that TSP-2's anti-tumor growth activity correlates with its ability to inhibit endothelial cell migration, rather than with a direct effect on growth or proliferation of tumor cells (see, e.g., page 33, lines 6-16; and page 35, line 15, to page 36, line 12). Further, the specification provides at least 2 assays that can be used to identify TSP-2 fragments that have the required endothelial cell migration inhibitory activity and/or tumor inhibition activity. For example, an *in vitro* human dermal microvascular endothelial cell (HDMEC) migration assay is described in detail at pages 39-40; and a xenograft tumor growth assay combined with a vessel density assay in nude mice is described at page 33, line 17, to page 34, line 19. The specification reports the use of the HDMEC assay to successfully identify active fragments. Indeed, the HDMEC migration assay alone was sufficient to identify a working example of an active TSP-2 fragment from only five fragments that were tested. In particular, applicants found that one of five (20%) of the TSP-2 fragments tested had HDMEC migration inhibitory activity of a level comparable to that of full length TSP-2 (47.6% inhibition by the peptide compared to 54.2% inhibition by full length TSP2) (see page 39, line 24 to page 40, line 15). That one of merely five tested fragments gave a positive result is clearly indicative of the routine nature of the assay.

at 10% what's changing?

As the Examiner is aware, the law does not require an applicant to describe in his specification every conceivable embodiment of the invention. It is sufficient that one embodiment is disclosed if other embodiments can be determined without undue experimentation. (See *U.S. v. Telectronics, Inc.*, 857 F.2d 778, 8 USPQ2d 1217 (Fed. Cir. 1988),

cert. denied, 490 U.S. 1046 (1989), holding that since one embodiment and the method to determine other embodiments was set forth in the specification, the specification was enabling, regardless of the great time and expense involved in such determination; see also MPEP §2164.06). Applicants' specification clearly meets this standard, as the method provided in the specification for determining the cell migration inhibitory activity of a TSP-2 fragment is routine and Applicants have shown it can be used with a high degree of success.

Moreover, as discussed in the enclosed declaration under 37 C.F.R. §1.132 of Dr. Michael Detmar, filed herewith, the inventors, using the methods described in the application, have identified another fragment of TSP-2 that works in the claimed methods. The inventors made an N-terminal fragment of human TSP-2 (hTSP-2/NTF), encoded by nucleotides 213-1883 of SEQ ID NO:1 (see Figure 1 of declaration), containing the procollagen homology domain and 3 type-1 repeats of TSP-2. The fragment was effective to inhibit the migration of HDMEC cells as shown by using the same HDMEC assay as described in the specification (see Figure 2 of the declaration). The fragment was also effective to inhibit angiogenesis and growth of squamous cell carcinoma in vivo in mice using the same A431 xenotransplant assay as described in the specification (see Figures 3-5 of the declaration). Thus, the evidence presented in the enclosed declaration of Dr. Detmar clearly shows that one of ordinary skill in the art could perform the claimed methods using the guidance provided in the specification. Accordingly, Applicants respectfully request that the rejection be withdrawn.

With respect to claim 63, the Examiner states that "there is no teaching in the specification indicating how to make or select for functional fragments of a procollagen domain." Claim 63 has been amended to recite that the fragment has the specific activity of inhibiting endothelial cell migration. This aspect of the rejection is respectfully traversed with regard to the amended claim. The specification explicitly provides that the N-terminal procollagen homology domain of TSP-2 refers to the domain encoded by nucleotides 294-1367 of TSP-2 (see page 28, lines 27-28). As discussed above, techniques of making and testing fragments of a known sequence for the recited activity require only routine experimentation using the methods described in the specification.

Written Description

Claims 1, 13-23, and 53-74 are rejected under 35 U.S.C. 112, first paragraph for written description issues. The Examiner states that the specification

does not disclose any evidence regarding the treatment of unwanted cell proliferation by administering a biologically active fragment of TSP-2, SEQ ID NO:2, 6-11, a functional fragment of a fragment comprising a procollagen domain, or a type I repeat. Likewise, it does not disclose the isolation of and assaying of the claimed polypeptide fragments to determine if it possesses the same biological activity as TSP-2. In addition, no other examples are disclosed that conveys to one of skill in the art that the applicant was in possession of the claimed fragments. There is no actual reduction to practice, sufficient descriptive information, such as definitive structural features, which are critical to polypeptide activity, or complete detailed description of the function of claimed invention indicating that the claimed polypeptide fragments were indeed isolated, produced, and assayed from the uses disclosed."

The rejection has been met by substantially narrowing the claims to require the TSP-2 or fragment thereof to be least 90% identical to SEQ ID NO:2 or a fragment thereof, and to have the specific function of inhibiting endothelial cell proliferation. New claims 85-87 require even more stringent structural limitations. Thus, the claims are no limited by specific structural and functional characteristics and are sufficiently described in the specification.

Applicants respectfully traverse the rejection insofar as it may be applied to the presently pending claims. Numerous fragments that fall within the claims are described in the specification. For example, the specification provides that a TSP-2 polypeptide used in the claimed methods "includes a domain that includes at least one, two or three type 1 repeat(s)" (see page 17, lines 29-30). In addition, the specification provides as follows:

For example, the peptide can include a PWAEW sequence (about amino acid residues 386 to 390 of SEQ ID NO:2), or the fragment can include a WSPWAEW sequence (about amino acids 384 to 390 of SEQ ID NO:2), or conservative substitutions of either sequence. (Page 18, lines 10-13)

Construct I expresses selectively the N-terminal procollagen domain of TSP-2 (nucleotides 294-1367), the region with the least homology to TSP-1. Construct 2 expresses, in addition, the type I repeats (nucleotides 294-1883) which contain several biologically active sites including two CSVTCG sequences that mediate binding to the CD36 receptor on endothelial cells. Construct 3 expresses the type I repeats (nucleotides 1383-1883) only. Construct 4 expresses the full-length

mature TSP-2 molecule, excluding the signal peptide (nucleotides 294-3755) which is provided in the expression vector. Such recombinant proteins can be used for the generation of monoclonal anti-TSP-2 antibodies, for the establishment of a human TSP-2 ELISA, and for the systemic treatment of experimental tumors. (page 28, line 27 to page 29, line 5, emphasis added)

Further (and contrary to the Examiner's assertion), Applicants have disclosed the isolation and assaying of the disclosed TSP-2 polypeptide fragments to determine if they possess the same inhibitory activity on endothelial cell migration as TSP-2. (See, e.g., page 39, line 24 to page 40, line 15). Since Applicants disclose a clear correlation between TSP-2's inhibitory effect on endothelial cell migration and ability to inhibit tumor growth *in vivo*, as discussed above, the disclosed fragments are fully representative of TSP-2 fragments encompassed by the full scope of the pending claims. Therefore, one of ordinary skill in the art would understand that Applicants were in possession of the claimed invention at the time of filing. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Rejections Under 35 U.S.C. §103(a)

Claims 1,6, 13-23, 53-59, and 63-74 are rejected as unpatentable over Panetti, et al. (1997) in view of Volpert et al. (1995), Ferrara (1995), Laherty et al. (1992), and LaBell, et al. (1992). The present claims are directed to a method of treating a subject having a disorder characterized by unwanted cell proliferation. The method includes administering TSP-2 or a fragment of TSP-2 having the ability to inhibit endothelial cell migration. The Examiner states:

Panetti, et al. teach in vitro inhibition of cell proliferation by TSP-1 and -2, but does not teach administration of TSP-2. However, Volpert, et al., teach the inhibition of angiogenesis by TSP-1 and -2 via administration in rat cornea. It would be obvious to administer TSP-2 to a subject to treat a disorder characterized by unwanted cell proliferation since Panetti et al. teach that cell proliferation may be inhibited by TSP-2 and Volpert et al. teach the inhibition of angiogenesis by the same administration. One would be motivated to combine the two teachings since [Panetti] et al. teach that cell proliferation is one component of angiogenesis and Volpert, et al. teach *in vivo* methods.

Furthermore, it would be obvious that VEGF would be inhibited since Ferrara teach that VEGF inhibition in tumor growth suppression (abstract). In view of the above teaching, one would be motivated to use SEQ ID NO:6-10 in the method as claimed since Laherty et al., teach SEQ DINO:6-8 and LaBell et al teach SEQ ID

NO:6 and 8-10 because it is the same sequence and thus will have the same function.

Applicants respectfully traverse this rejection with regard to the presently pending claims. To establish *prima facie* obviousness of a claimed invention, the prior art must teach or suggest all the limitations of the claims, and the motivation to arrive at the present invention and a reasonable expectation of success must be found in the prior art. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991). In this instance, a *prima facie* case of obviousness has not been made because the cited references, alone or in combination, fail to disclose or suggest all the limitations of the present claims. Nor does the art provide a motivation or reasonable expectation of success for a skilled artisan to arrive at the presently claimed methods. In fact, the art teaches away from the present claims, as discussed below.

Panetti et al. teach that TSP-2 inhibits endothelial cell proliferation in an *in vitro* assay for thymidine incorporation. Panetti says nothing about endothelial cell migration, as recited in the present claims and does not disclose or suggest administering TSP-2 to a subject having a cell proliferative disorder. Volpert discloses that TSP-2 inhibits neo-vascularization in rat cornea, but also does not disclose or suggest administering TSP-2 or a fragment thereof to inhibit tumor growth in a subject. The Examiner states that "one would be motivated to combine the two teachings since [Panetti] et al. teach that cell proliferation is one component of angiogenesis and Volpert, et al. teach *in vivo* methods."

This rejection is respectfully traversed. The MPEP plainly states that "the mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination." MPEP § 2143.01. The Examiner's statement of motivation to combine the references is conclusory and not supported by the cited references. None of the cited references, alone or in any combination, disclose or suggest the use of TSP-2 or a fragment thereof, to treat a cell proliferative disorder. Rather than point to TSP-2 as a treatment for cell proliferative disorders, the prior art is negative, or at best, indifferent, about such a role for TSP-2. The cited art focuses on TSP-1, rather than TSP-2, as being the more potent and desirable TSP for inhibition of cell proliferation. For example, Panetti provides that "TSP2 concentrations [to inhibit endothelial cell proliferation] were required that

are approximately 10 times greater than those required for TSP1" (Panetti, page 212, emphasis added). Similarly, Volpert teaches that "all inducers [of angiogenesis] were efficiently inhibited by TSP-1 and most were also inhibited by TSP-2, although TSP-2 was less effective against VEGF and almost ineffective against TGFB" (Volpert, page 329, emphasis added). Therefore, if anything, one of ordinary skill in the art would interpret the much lower potency of TSP-2 compared to TSP-1 for inhibiting angiogenesis, as a suggestion that TSP-2 is in fact not desirable for the treatment of a cell proliferation disorder.

Ferrara discloses the role of VEGF in angiogenesis. Ferrara does not mention TSP-2. Laherty et al. and LaBell et al. each disclose the sequence of TSP-2. None of these references, alone or in combination, cure the deficiencies of the primary references of Panetti and Volpert. If anything, Laherty teaches away from the claimed methods. Laherty discloses that TSP2 "is expressed during cell proliferation and embryogenesis" (see Laherty, page 3280, right column). Clearly, if the expression of TSP-2 is associated with cell proliferation, one would hardly expect that administering TSP-2 would be effective to treat a disorder of cell proliferation. To the contrary, the disclosure of Laherty would motivate one to decrease TSP-2 rather than increase it in order to treat a cell proliferative disorder. For at least these reasons, the present claims are not obvious over the cited references, alone or in combination.

In light of the foregoing, Applicants respectfully request that the rejection be withdrawn.

Attached is a marked-up version of the changes being made by the current amendment.

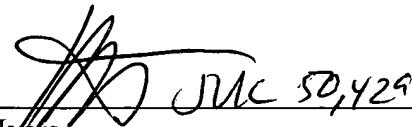
Applicant : Michael J. Detmar et al.
Serial No. : 09/536,087
Filed : March 24, 2000
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Applicant asks that all claims be allowed. Enclosed is a check for excess claim fees and a Petition for Extension of Time fee with the required fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: October 1, 2002



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Version with markings to show changes made

In the specification:

Paragraph beginning at page 26, line 1 has been amended as follows:

Figure 3 is a [graft]graph showing that transfected TSP-2 inhibits intradermal tumor growth of A431 squamous cell carcinoma cells (left) and MeWo malignant melanomas (right).

Paragraph beginning at page 26, line 4 has been amended as follows:

Figure 4 [are grafts] depicts graphs showing the effects of TSP-2 on tumor angiogenesis, the average vessel density (Figure 4A), vessel size (Figure 4B), the number of vessels found in the size range of less than 500 μm^2 and larger than 1500 μm^2 (Figure 4C), and the percentage of tissue area covered by vessels (Figure 4D). |

Paragraph beginning at page 26, line 12 has been amended as follows:

Figure 6 is a [graft] graph showing the migration of human dermal microvascular endothelial cells (HDMEC) and the effect of TSP-1 (T1) or TSP-2 (T2) binding of the CD36 receptor on the migration of these cells. HDMEC were incubated alone (C), in the presence of TSP-1 (T1) or TSP-2 (T2), or in the presence of TSP-1 (T1) or TSP-2 (T2) in the presence of an anti-CD36 antibody (36). |

Paragraph beginning at page 26, line 17 has been amended as follows:

Figure 7 is a [graft] graph showing the effect of HDMEC migration in the presence of various synthetic TSP-2 derived peptides. Peptides 1, 2, 3 and 4 (P1, P2, P3, P4) (SEQ ID NOs:6-9, respectively) were derived from the procollagen domain of TSP-2, peptide 7 (P7) (SEQ ID NO:10) was derived from the first type 1 repeat of TSP-2. |

In the claims:

Claims 62 and 69-74 have been cancelled.

Claims 1, 18, 58, 60 and 63-67 have been amended as follows:

1. (Amended) A method of treating a subject having a disorder characterized by unwanted cell proliferation, the method comprising administering to the subject a TSP-2 comprising an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:2, or a [biologically active] fragment thereof capable of inhibiting endothelial cell migration.

18. (Amended) The method of claim 1, wherein the disorder is characterized by benign unwanted skin proliferation [in the skin].

58. (Amended) The method of claim 1, wherein the fragment comprises at least one type I repeat [or functional fragment thereof].

60. (Amended) The method of claim 1, wherein the fragment comprises at least one sequence selected from the group of: amino acids 382-429 of SEQ ID NO:2, amino acids 438-490 of SEQ ID NO:2, and amino acids 495-547 of SEQ ID NO:2[, or a functional fragment thereof].

63. (Amended) The method of claim 1, wherein the fragment comprises a procollagen domain or a [functional] fragment thereof having the ability to inhibit endothelial cell migration.

64. (Amended) The method of claim [63] 1, wherein the fragment comprises SEQ ID NO:6.

65. (Amended) The method of claim [63] 1, wherein the fragment comprises SEQ ID NO:7.

66. (Amended) The method of claim [63] 1, wherein the fragment comprises SEQ ID NO:8.

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67. (Amended) The method of claim [63] 1, wherein the fragment comprises SEQ ID NO:9.